[0031] FIG. **3** is a schematic drawing illustrating a molecular linker composed of dsDNA and PEG.

[0032] FIGS. **4** A-F are graphs showing the distance between fluorophores and FRET at several molecular rod lengths. A rod has two tethers and FRET is measured between the tether tips. This is a computer simulation. The tethers are 120 Å long and consist of segments that have the persistence length of PEG (3.8 Å). (E and F) rod length 0 Å. (C and D) rod length 60 Å. (A and B) rod length 120 Å. The data were generated using the bite program and graphed using the genhis and genpic programs.

[0033] FIGS. **5**A-D are graphs showing the effect of tether length on FRET at various rod lengths. The FRET distance, R_0, is 60 Å. Each graph shows the FRET efficiency versus the rod length. The color corresponds to the frequency that the nanoprobe gives a particular FRET signal. (A) tether length 2 Å; (B) tether length 60 Å; (C) tether length 120 Å; (D) tether length 240 Å. The data of the graphs of FIG. **4** can be obtained from the lower left part of FIG. **5** (such as FIG. **5**C, with a tether length of 120 Å) by taking vertical slices at rod lengths of 0 Å, 60 Å and 120 Å. The data were generated using the bite program and graphed using programs genhis and denplo.

[0034] FIG. **6** is a trace showing an example result for a target sequence.

[0035] FIG. **7** is a schematic drawing illustrating (top) a hairpin oligonucleotide having a 5' overhang and fluorescent donor label (circle), and a 3' dideoxynucleotide. A freely diffusing dTTP is labeled with a FRET acceptor (hexagon). The labeled dTTP can bind to the first base of the overhang, but the dTTP cannot be incorporated into the oligonucleotide. The bottom shows FRET between the donor and acceptor when the labeled dTTP is held to the hairpin by a polymerase (ellipse). This dwell can be measured using methods known in the art, for example using fluorescence correlation spectroscopy (FCS).

[0036] FIG. **8** is a schematic drawing illustrating (top) a hairpin oligonucleotide ending with a dideoxynucleotide at the 3' end and having a donor fluorophore near the 5' end. An acceptor-labeled dTTP is attached via a PEG tether. Although the first base in the overhang is an A, the tethered dTTP will not stay close to the "A" and little or no FRET should occur. The bottom shows that when a DNA polymerase (ellipse) binds to the DNA hairpin, the tethered dTTP should dwell in the enzyme-DNA pocket, allowing FRET between the two fluorophores.

SEQUENCE LISTING

[0037] The nucleic acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases. In particular examples, only one strand of a nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand (for example in the case of a dsDNA molecular rod).

[0038] SEQ ID NO: 1 is an exemplary target sequence.

[0039] SEQ ID NO: 2 is the compressed version of SEQ ID NO: 1.

[0040] SEQ ID NOS: 3-26 are sequences that can be used to generate the probe shown in FIG. **2**C.

[0041] SEQ ID NOS: 27-30 are sequences that can be substituted for SEQ ID NOS: 3, 5, 7, and 9, respectively.

[0042] SEQ ID NOS: 31-38 are sequences that can form hairpin loops.

[0043] SEQ ID NO: 39 is an exemplary target sequence.

DETAILED DESCRIPTION OF SEVERAL EMBODIMENTS

Abbreviations and Terms

[0044] The following explanations of terms and methods are provided to better describe the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure. As used herein, "comprising" means "including" and the singular forms "a" or "an" or "the" include plural references unless the context clearly dictates otherwise. For example, reference to "a molecular linker" includes one or a plurality of such molecular linkers, and reference to "the probe" includes reference to one or more probes and equivalents thereof known to those skilled in the art, and so forth. The term "or" refers to a single element of stated alternative elements or a combination of two or more elements, unless the context clearly indicates otherwise. For example, the phrase "a tether or a molecular rod" refers to one or more tethers, one or more molecular rods, or a combination of both one or more tethers and one or more molecular rods.

[0045] Unless explained otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. The materials, methods, and examples are illustrative only and not intended to be limiting. Other features and advantages of the disclosure are apparent from the following detailed description and the claims.

Å	angstrom
dsDNA	double-stranded DNA
FRET	Förster resonance energy transfer
GFP	green fluorescent protein
LNA	locked nucleic acid
PEG	polyethylene glycol
PNA	peptide nucleic acid
RT	reverse transcriptase
ssDNA	single-stranded DNA

[0046] Acceptor fluorophore: Compounds which absorb energy from a donor fluorophore, for example in the range of about 400 to 900 nm (such as in the range of about 500 to 800 nm). Acceptor fluorophores generally absorb light at a wavelength which is usually at least 10 nm higher (such as at least 20 nm higher), than the maximum absorbance wavelength of the donor fluorophore, and have a fluorescence emission maximum at a wavelength ranging from about 400 to 900 nm. Acceptor fluorophores have an excitation spectrum which overlaps with the emission of the donor fluorophore, such that energy emitted by the donor can excite the acceptor. Ideally, an acceptor fluorophore is capable of being attached to the disclosed nanoprobes.

[0047] Exemplary acceptor fluorophores include, but are not limited to, rhodamine and its derivatives (such as N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), 6-carboxy-X-rhodamine (ROX)), fluorescein derivatives (such as 5-carboxyfluorescein (FAM) and 2'7'-dimethoxy-4'5'-dichloro-6-